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Patterns of Morphological and Molecular Evolution in the Antillean Tree Bat, *Ardops nichollsi* (Chiroptera: Phyllostomidae)

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Abstract

Species endemic to oceanic islands offer unique insights into the mechanisms underlying evolution and have served as model systems for decades. Often these species show phenotypic variation that is correlated with the ecosystems in which they occur and such correlations may be a product of genetic drift, natural selection, and/or environmental factors. We explore the morphologic and genetic variation within *Ardops nichollsi*, a species of phyllostomid bat endemic to the Lesser Antillean islands. *Ardops nichollsi* is an ideal taxon to investigate the tempo of evolution in Chiroptera, as it: is a recently derived genus in the family Phyllostomidae; contains intraspecific morphological variation; and has a restricted insular distribution. To evaluate patterns of evolution in *A. nichollsi*, we used standard morphological analyses, in addition to analyzing Amplified Fragment Length Polymorphisms, mitochondrial cytochrome-*b*, and paternal marker zinc finger Y-chromosomal intron DNA sequence data. Our results identified a pattern that consists of two distinct evolutionarily lineages, which correspond to northern and southern islands of the Lesser Antilles. We also describe a new subspecies from the southern island of Saint Vincent. These results indicate gene flow among northern Lesser Antillean populations during the Pleistocene, and local adaptation to individual islands in the southern Lesser Antilles. Our findings can be used to further explore speciation processes within Caribbean bats and, more broadly, within species distributed across other insular systems.

Key words: AFLP, *Ardops*, *Ariteus*, Caribbean, incipient species, island biogeography, Lesser Antilles, speciation, subspecies

Introduction

Studies of species endemic to oceanic archipelagos have offered important insights into mechanisms of evolution, and have advanced evolutionary theory (Darlington 1957; MacArthur and Wilson 1967; Heaney 2007; Losos and Ricklefs 2010). Species complexes such as Caribbean *Anolis* and Darwin's Galapagos finches are considered to be model organisms and provide a foundation for understanding natural selection, adaptation, colonization, and speciation of island fauna (Roughgarden and Roughgarden 1995; Hedges 1996; Grant and Grant 2008; Pinto et al. 2008). It is within this framework that we approach *Ardops nichollsi*,

a Lesser Antillean endemic. The monotypic genus *Ardops* is of relatively recent origin, between 1.8–2.0 million years ago (mya; Rojas et al. 2011; Baker et al. 2012), making it one of the youngest lineages of the phyllostomid bats. Interestingly, *A. nichollsi* exhibits a relatively large amount of intraspecific morphological variation (Jones and Schwartz 1967; Jones and Genoways 1973). Given its insular distribution, the foregoing means that it successfully dispersed, established island populations, and then adapted to local environments. Included within the *A. nichollsi* complex are five morphologically defined subspecies (*montserratensis*, *annectens*, *nichollsi*, *koopmani*, *luciae*; Fig. 1), which exhibit variation in body size across the 13 Lesser Antillean islands they inhabit (Jones and Schwartz 1967; Masson et al. 1990; McCarthy and Henderson 1992;

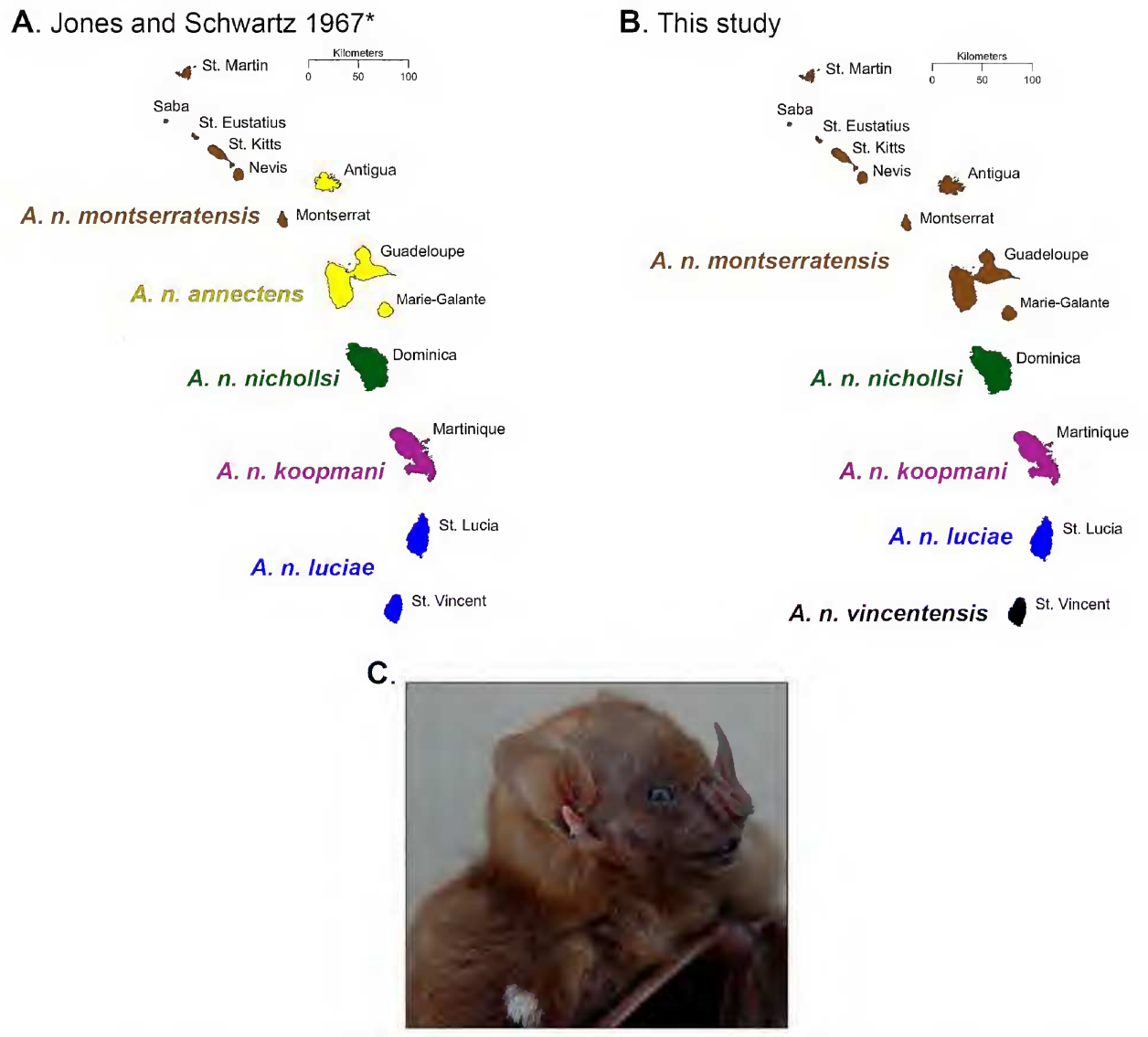

Figure 1. Map of the Lesser Antilles showing geographic variation in *Ardops nichollsi*. (A) Distribution based on previous morphological analyses (*sensu Jones and Schwartz 1967; Jones and Genoways 1973; Genoways et al. 2007a; Lindsay et al. 2010). Color coding: *A. n. montserratensis* – brown; *A. n. annectens* – yellow; *A. n. nichollsi* – green; *A. n. koopmani* – purple; and *A. n. luciae* – blue. (B) Current taxonomy and distribution based on this study. Color coding: *A. n. montserratensis* – brown; *A. n. nichollsi* – green; *A. n. koopmani* – purple; *A. n. luciae* – blue; and *A. n. vincentensis* – black. (C) Photograph of *Ardops nichollsi* from St. Vincent (by P. A. Larsen).

Pedersen et al. 2003, 2005; Genoways et al. 2001, 2007a, 2007b; Lindsay et al. 2010).

Geographically defined phenotypes are typically classified as subspecies, but empirical studies have questioned whether or not such geographically or ecologically defined units represent the initial stages of speciation (Mayr 1954; Pimentel 1958; Lidicker 1962; Baker and Bradley 2006; Johnsen et al. 2006; Patten and Pruett 2009). Here, we consider the variation within *Ardops* as an appropriate system to begin formulating hypotheses regarding incipient speciation given the hypothesized time of origin for *Ardops* is recent, and the potential for unique evolutionary pressures associated with an archipelago (i.e., divergent selection, founder effects, and others).

Jones and Schwartz (1967) conducted the first detailed examination of the variation within *A. nichollsi*. Based on cranial and external measurements, these authors identified a continuum in size and extensive secondary sexual variation within the genus, hypothesizing the populations adapted independently to environmental conditions on each island. Since then, few studies have specifically explored relationships within *A. nichollsi* or closely related genera (Greenbaum et al. 1975; Mennone et al. 1986; Carstens et al. 2004; Davalos 2007; Baker et al. 2012), with each of these studies lacking specimens from throughout the known distribution. However, our expeditions to the Lesser Antilles have increased the number of available voucher specimens and tissues of *A. nichollsi* (Pedersen et al. 2003, 2005; Genoways et al. 2001, 2007a, 2007b, 2010; Lindsay et al. 2010). To better understand the evolutionary history of *A. nichollsi*, we analyzed nuclear, mitochondrial, and Y-chromosomal markers in addition to morphological characters from specimens collected throughout the Lesser Antilles. These data allow for an analysis of genetic and phenotypic variation within the genus and for the formulation of hypotheses concerning the delimitation of subspecies, and incipient speciation in an archipelago.

MATERIALS AND METHODS

Molecular methods.—Tissues were collected from natural populations of *Ardops nichollsi* throughout the Lesser Antilles and *Ariteus flavescens* from Jamaica (outgroup and sister genus representative; Appendix). Whole genomic DNA was extracted from liver or muscle tissue for all genetic analyses following standard methods (Longmire et al. 1997), or by using DNeasy Blood and Tissue Kits (Qiagen Inc., Chatsworth, California). All tissues used in DNA analyses are archived at the Genetic Resources Collection of the Natural Science Research Laboratory (NSRL) of Texas Tech University. All animals were handled following the guidelines for animal care and use established by the American Society of Mammalogists (Sikes et al. 2011; Texas Tech University IACUC permits #02217-02 and #07083-02).

External primers glo7L/glo6H (Hoffmann and Baker 2001) were used to amplify 1,140 base pairs (bp) of the cytochrome *b* (cyt-*b*) gene in three *Ariteus* and 47 *Ardops* specimens. The thermal profile consisted of 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 49°C for 1 min, extension at 72°C for 1 min 15 s, and ended with 72°C for 10 min. All PCR products were purified using QIAquick PCR Purification Kits (Qiagen Inc., Chatsworth, California). Internal primers from Hoffmann and Baker (2001; glo1L, glo5L), Smith and Patton (1991; MVZ 04), and Larsen et al. (2007b; ART16) were used to obtain final sequences of the cyt-*b* gene. While sequencing cyt-*b*, we discovered the inadvertent amplification of a pseudogene (translocation of a mitochondrial gene into the nuclear genome) for two specimens (TK 15576, TK 129202). To obtain the full cyt-*b* gene sequence from the mitochondrial genome for these samples, two long-range primers (Art563_32merF [5′-GGT-ATG-GGC-CCG-ATA-GCT-TAT-TTA-GCT-GAC-CT-3′]; Art765_32merR [5′-ATG-ACC-AAC-ATT-CGA-AAA-ACT-CAC-CCC-TTA-TT-3′]) were developed (CDP) and used to amplify a 6.3 kilo-base fragment of the mitochondrial genome (NADH dehydrogenase 1 through cyt-*b*). The complete cyt-*b* gene subsequently was sequenced from these amplicons.

Primers from Cathey et al. (1998; LGL335F and LGL331R) were used to amplify and sequence 969 bp of an intron of the zinc finger Y-chromosome gene (ZFY) from two male *Ariteus flavescens* and 22 male

Ardops nichollsi. The thermal profile consisted of 95°C for 3 min, followed by 32 cycles of denaturation at 95°C for 45 s, annealing at 50°C for 30 s, extension at 70°C for 2 min 30 s, and ended with 70°C for 5 min. PCR products were electrophoresed on a 0.8% agarose gel and excised using Qiagen Gel Extraction Kits (Qiagen Inc., Chatsworth, California). Samples were prepared for sequencing with Centri-Sep columns (Princeton Separations, Freehold, New Jersey). DNA sequencing for the cyt-*b* and ZFY genes was performed using ABI Big Dye v3.1 chemistry, and fragments were electrophoresed on an ABI 3100-Avant Genetic Analyzer (PE Applied Biosystems, Foster City, California). Sequences were verified and assembled using Sequencher v4.10.1 (Gene Codes Corporation, Ann Arbor, Michigan). To ensure correct open-reading frame, multiple sequence alignments were performed manually and further checked in MacClade v4.08 (Maddison and Maddison 2005) and/or Clustal W (Larkin et al. 2007).

AFLPs were generated from three *Ariteus* and 47 *Ardops*, following the protocols of Vos et al. (1995) and McDonough et al. (2008). A labeled (6FAM fluorophore; Applied Biosystems, Foster City, California) selective EcoRI primer and six non-labeled selective primers (McDonough et al. 2008) were used to generate AFLPs from *Ariteus flavescens* and *Ardops nichollsi*. The labeled fragments were detected using an ABI 3100-Avant genetic analyzer, scored for presence or absence using GeneMapper v4.0 (Applied Biosystems), and converted into a binary data matrix using GenAlEx v6.5 (Peakall and Smouse 2012). Only fragments (50–400 bp in length) with intensities larger than 100 relative fluorescence units were scored as present. Error rates (technical error rate and observer error rate) were obtained following Bonin et al. (2004) using 26 replicated samples (approximately 9% of the overall sample size).

Molecular analyses.—Phylogenetic analyses of cyt-*b* sequence data were performed using MEGA v5.2 (Tamura et al. 2011), MrBayes v3.2 (Ronquist et al. 2012), and Garli v2.0 (Zwickl 2006) software. Maximum-parsimony, Bayesian, and maximum-likelihood analyses were used to infer cyt-*b* phylogenies. Maximum-parsimony analyses were performed using heuristic searches, 25 replicates of the random taxon addition option, each with random starting trees, and tree-bisection-reconnection branch swapping. Substitution models of DNA evolution were analyzed in MEGA to determine the appropriate model for the cyt-*b* gene sequence data and the HKY+G model was chosen. Maximum-likelihood analyses were performed in Garli using 100 search replicates, with searches being terminated after the last topological improvement following 5×10^6 generations. Bootstrap support values for maximum-parsimony and maximum-likelihood analyses were calculated based on 500 iterations and values $\geq 75\%$ were considered statistically supported. Bayesian analyses were performed using 5 million generations (1 cold and 3 incrementally heated Markov chains, random starting trees for each chain), and trees were sampled every 100 generations with a final 25% burn-in (convergence was confirmed using Tracer v1.5; Rambaut and Drummond 2007). Posterior probabilities ≥ 0.95 were considered statistically supported.

Genetic distance values for cyt-*b* were calculated in MEGA using the Kimura 2-parameter model (Kimura 1980), which allowed for comparisons with previous molecular studies of *Ardops* (Carstens et al. 2004) and with other mammals (Bradley and Baker 2001; Baker and Bradley 2006). Based on previous molecular studies, *A. flavescens* is an appropriate outgroup for the phylogenetic analyses (Baker et al. 2000, 2003, 2012; Davalos 2007). Additional cyt-*b* sequences of *Ardops* were obtained from GenBank and included in the analyses (Carstens et al. 2004; see Appendix: HapA–I). The paternal ZFY gene sequence data was highly conserved (see Results; Table 1) and as such was analyzed via sequence alignment.

Bayesian clustering of AFLP data was performed using STRUCTURE v2.3 (Pritchard et al. 2000) and GENELAND v4.0 (Guillot et al. 2005). STRUCTURE analyses were performed using ten iterations of 200,000 replications with a burn-in of 50,000 for each iteration, allowing for admixture with correlated allele frequencies. Analyses were streamlined using the StrAuto v0.3 python utility by Chhatre (2012). Our sample of *Ardops* consisted of individuals collected from eight islands, thus our K (cluster) values ranged from 1 to 8 for each STRUCTURE iteration. Structure Harvester v0.6 (Earl and vonHoldt 2011) was used to implement the delta K procedure of Evanno et al. (2005), where the estimated number of clusters was chosen based on the greatest Pr(X|K). GENELAND was used for spatial analyses of AFLP data with 1,000,000 itera-

Table 1. Characters in the ZFY sequence data. Base pair references are given above the sequences. "…" indicates removed sequence positions that contain no polymorphisms. For *Ardops nichollsi*, islands are listed from north to south (the line delineates northern from southern islands). *Ariteus flavescens* is only known from Jamaica (delineated by double line). Each island group (north and south) is represented by a single haplotype. Sample sizes (n) from each island are listed.

Island	n	…	21	22	23	24	25	26	27	…	80	81	82	83	84	85	86	…	92	93	94	95	96	97	98	…
St. Eustatius	4	…	C	A	T	T	G	T	A	…	G	T	T	A	T	G	T	…	C	T	A	G	T	T	A	…
St. Kitts	1	…	–	–	–	–	–	–	–	…	–	–	–	–	–	–	–	…	–	–	–	–	–	–	–	…
Montserrat	2	…	–	–	–	–	–	–	–	…	–	–	–	–	–	–	–	…	–	–	–	–	–	–	–	…
Dominica	4	…	–	–	–	–	–	–	–	…	–	–	–	–	–	–	–	…	–	–	–	–	–	–	–	…
St. Lucia	7	…	–	–	–	C	–	–	–	…	–	–	–	G	–	–	–	…	–	–	–	A	–	–	–	…
St. Vincent	4	…	–	–	–	C	–	–	–	…	–	–	–	G	–	–	–	…	–	–	–	A	–	–	–	…
Jamaica	2	…	–	–	–	C	–	–	–	…	–	–	–	G	–	–	–	…	–	–	–	A	–	–	–	…

Island	n	…	160	161	162	163	164	165	166	…	582	583	584	585	586	587	588	…
St. Eustatius	4	…	A	A	T	G	A	T	A	…	T	T	T	A	A	A	A	…
St. Kitts	1	…	–	–	–	–	–	–	–	…	–	–	–	–	–	–	–	…
Montserrat	2	…	–	–	–	–	–	–	–	…	–	–	–	–	–	–	–	…
Dominica	4	…	–	–	–	–	–	–	–	…	–	–	–	–	–	–	–	…
St. Lucia	7	…	–	–	–	T	–	–	–	…	–	–	–	T	–	–	–	…
St. Vincent	4	…	–	–	–	T	–	–	–	…	–	–	–	T	–	–	–	…
Jamaica	2	…	–	–	–	T	–	–	–	…	–	–	–	T	–	–	–	…

Island	n	…	708	709	710	711	712	713	714	…	811	812	813	814	815	816	817	…
St. Eustatius	4	…	G	A	G	C	T	G	G	…	T	G	T	G	T	G	T	…
St. Kitts	1	…	–	–	–	–	–	–	–	…	–	–	–	–	–	–	–	…
Montserrat	2	…	–	–	–	–	–	–	–	…	–	–	–	–	–	–	–	…
Dominica	4	…	–	–	–	–	–	–	–	…	–	–	–	–	–	–	–	…
St. Lucia	4	…	–	–	–	T	–	–	–	…	–	–	–	A	–	–	–	…
St. Vincent	2	…	–	–	–	T	–	–	–	…	–	–	–	A	–	–	–	…
Jamaica	2	…	–	–	–	T	–	–	–	…	–	–	–	A	–	–	–	…

tions, 100 thinning intervals, and a burn-in of 25%. The correlated allele frequencies model was used and the maximum population number was set to eight. We performed three runs to test for consistency for the number of populations estimated by GENELAND and we selected the run with the highest average posterior probability. Additional analyses of AFLP data included a principal coordinates analysis (PCoA) and an analysis of molecular variance (AMOVA), both of which were conducted using GenAlEx v6.5 (Peakall and Smouse 2012). PhiPT analyses were conducted in GenAlEx with 9,999 permutations, with PhiPT (analogous to F_{ST}) representing the proportion of variance among populations relative to the total variance.

Morphological methods.—Voucher specimens are located at the following institutions: American Museum of Natural History (AMNH); British Museum of Natural History (BMNH); Royal Ontario Museum (ROM); Texas Tech University (TTU); and University of Nebraska State Museum (UNSM). A total of 52 females and 48 males of *Ardops nichollsi* (indicated by ♀ and ♂ symbols in Appendix) were used in morphological analyses. Following the definitions and methods of Hall (1946) and Genoways et al. (2007b), seven cranial measurements were recorded from adult specimens of *Ardops nichollsi* from throughout their geographic distribution (based on criteria from Kunz and Anthony 1982). Measurements were taken from museum specimens using digital calipers and are given in millimeters to the nearest 0.01 mm. Measurements include: GLS = greatest length of skull; CBL = condylobasal length; ZB = zygomatic breadth; POC = postorbital constriction; MB = mastoid breadth; MTR = maxillary toothrow length; and MM = breadth across upper molars.

Statistical analyses tested for secondary sexual dimorphism (one-way multivariate analysis of variance, MANOVA) and evaluated the extent of morphological variability in our sample of the *Ardops nichollsi* complex (principal component analysis). Since the number of subspecies in *A. nichollsi* has been debated (Jones and Genoways 1973), a conservative approach of analyzing the data as one unit was utilized. Principal component analysis (PCA) does not take into account any difference between groups based on apriori classification of the sample. Overall, variation in cranial size was summarized by the first axis of the PCA (PC1). Loadings are reported to describe the direction and magnitude of measurements with their respective axis. Descriptive statistics (mean, standard deviation, and range) were obtained for all individuals from each island. Statistical analyses were performed in R statistical software (2014).

RESULTS

Fifty sequences of the mitochondrial cyt-*b* gene and 289 AFLP bands were generated from *Ardops* and *Ariteus* (outgroup and sister genus representative from Jamaica). For each AFLP primer pair, an average of 50 bands were scored. An error rate of 1.0% (3 bands of 298) was estimated, with these discrepancies originating from poor amplification. Additionally, ZFY sequence data were generated from 24 males (2 *Ariteus* and 22 *Ardops*). Cyt-*b* and ZFY sequences were deposited in GenBank and accession numbers for all DNA sequence data herein are presented in the specimens examined (Appendix).

Phylogenetic analyses.—Sequence alignment of the complete cyt-*b* gene from 47 *Ardops* (in addition to the nine individuals of *Ardops* from Carstens et al. 2003) and three *Ariteus* was unequivocal and without stop codons. Including the outgroup, 110 sites were variable with 79 being parsimony-informative with seven at codon position 1, three at position 2, and 69 at position 3. Maximum-likelihood analyses resulted in a single optimal tree (-lnL = 2311.91); nucleotide frequencies of A = 0.292, C = 0.326, G = 0.123, and T = 0.259; a transition/transversion ratio of 10.09; and an alpha shape parameter of the gamma distribution of 0.867. Tree topologies resulting from maximum-parsimony, Bayesian, and maximum-likelihood analyses were largely congruent with differences arising at unsupported nodes. The monophyly of *A. nichollsi* was statistically supported; however only four clades within the species had statistical support and these did not strictly correspond to island occurrence (Fig. 2). The level of intraspecific variation among cyt-*b* sequences was found to be $\leq$ 1.0% in *A. nichollsi*, whereas the

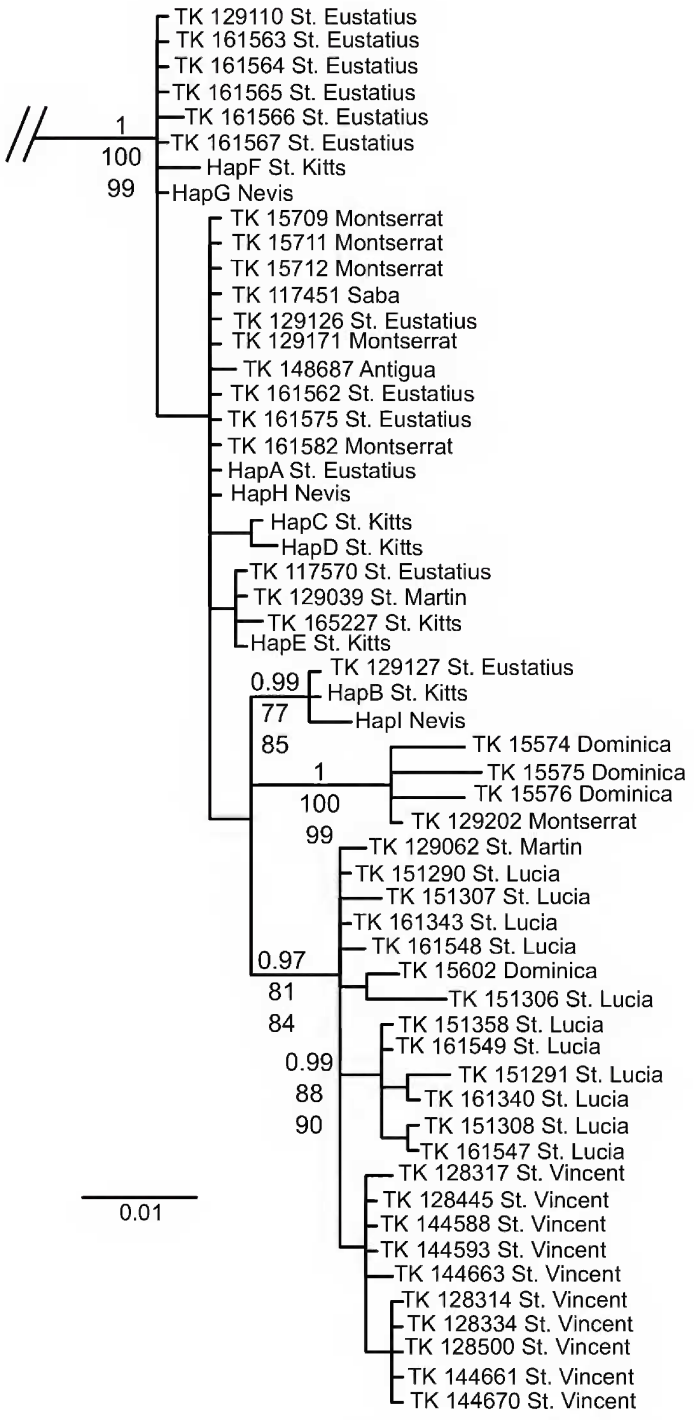

Figure 2. Bayesian phylogram of 1140 base pairs of the cyt-*b* gene from 59 individuals. (*Ariteus flavescens* was used as an outgroup but is not shown.) Top score = Bayesian posterior probability, middle score = maximum likelihood bootstrap, bottom score = maximum parsimony bootstrap. Bootstrap support values are percentages of 500 iterations. Values for unsupported nodes are not shown.

sequences of *Ariteus* averaged ~ 5.0% divergent from *Ardops*.

The ZFY intron sequenced from male *Ardops nichollsi* was highly conserved across all individuals (Table 1). However, specimens collected from St. Lucia and St. Vincent were found to have the same ZFY intronic sequence as the outgroup *Ariteus flavescens* (currently distributed only on Jamaica). Males of *A. nichollsi* from northern Lesser Antillean islands (St. Eustatius, St. Kitts, Montserrat, and Dominica) differed from the outgroup at seven nucleotide positions (5 transitions and 2 transversions; Table 1).

AFLP analyses.—Our STRUCTURE analyses of 47 *Ardops* resulted in the identification of two clusters or groups within the AFLP sample of *A. nichollsi* (Figs. 3A, B). The blue group (Fig. 3B) included individuals collected from throughout the northern Lesser Antilles (Montserrat, Saba, St. Eustatius, St. Kitts, St. Martin, and Dominica), whereas the red group (Fig. 3B) was comprised of individuals collected from St. Lucia and St. Vincent. Alternatively, GENELAND identified three groups within the AFLP data (Fig. 3C: corresponding to northern Lesser Antilles, St. Lucia, and St. Vincent, respectively). The principal coordinates analysis (PCoA) of AFLP genetic distance (Fig. 4) reinforced the GENELAND results, with three groups also corresponding to the northern Lesser Antilles, St. Lucia, and St. Vincent. The first, second, and third principal coordinates accounted for 39.32%, 31.91%, and 11.76% of the total variation, respectively. An analysis of molecular variance (AMOVA) of the three groups identified with GENELAND and PCoA resulted in 51% of the total variance being observed among populations and 49% within (Table 2). Pairwise PhiPT values were 0.53 (northern Lesser Antilles versus St. Lucia), 0.55 (northern Lesser Antilles versus St. Vincent), and 0.35 (St. Lucia versus St. Vincent).

Morphological analyses.—Secondary sexual variation was significant ($P < 0.001$) for each variable among the sampled *Ardops*; therefore the sexes were separated in further statistical analyses. The PCA of females revealed overlap among individuals from Montserrat, St. Kitts, St. Eustatius, Guadeloupe, Martinique, Antigua, and St. Lucia, whereas Dominica and St. Vincent were separate and distinct along the first principal component. For females, PC1 explained 81.7% of the variation and PC2 explained 8.3% of the variation (Fig. 5A). The variables that had the highest component loading on PC1 for females were ZB, MB, MTR, and MM (Table 3); however, all seven variables were correlated with PC1 to a similar extent and in the same direction (Fig. 5A). POC showed a very high component loading with PC2. The PCA of males revealed overlap among individuals from Montserrat, St. Kitts, St. Eustatius, Guadeloupe, Martinique, Nevis, Antigua, and St. Lucia, whereas Dominica and St. Vincent were separate and distinct along the first principal component. For males, PC1 explained 87.2% of the variation and PC2 explained 5.0% of the variation (Fig. 5B). The variables that had high component loadings on PC1 for males were ZB, MTR, and MM (Table 3); however, all seven variables were correlated with PC1 to a similar extent and in the same direction just as was seen in females (Fig. 5B). PC2 had high component loadings, but they were mixed (in opposite directions) for POC and MB (Table 3). Overall, the variance along PC1 in males and females was primarily due to cranial size, and all measurements from individuals from the northern end of the range and from St. Lucia were larger, whereas those from Dominica and St. Vincent were generally smaller. On PC2, POC appeared to explain most of the variation within males and within females; however MB is also important in the variation seen in males on PC2. Mean, standard deviation, and range are reported for individuals by island for each sex (Tables 4, 5).

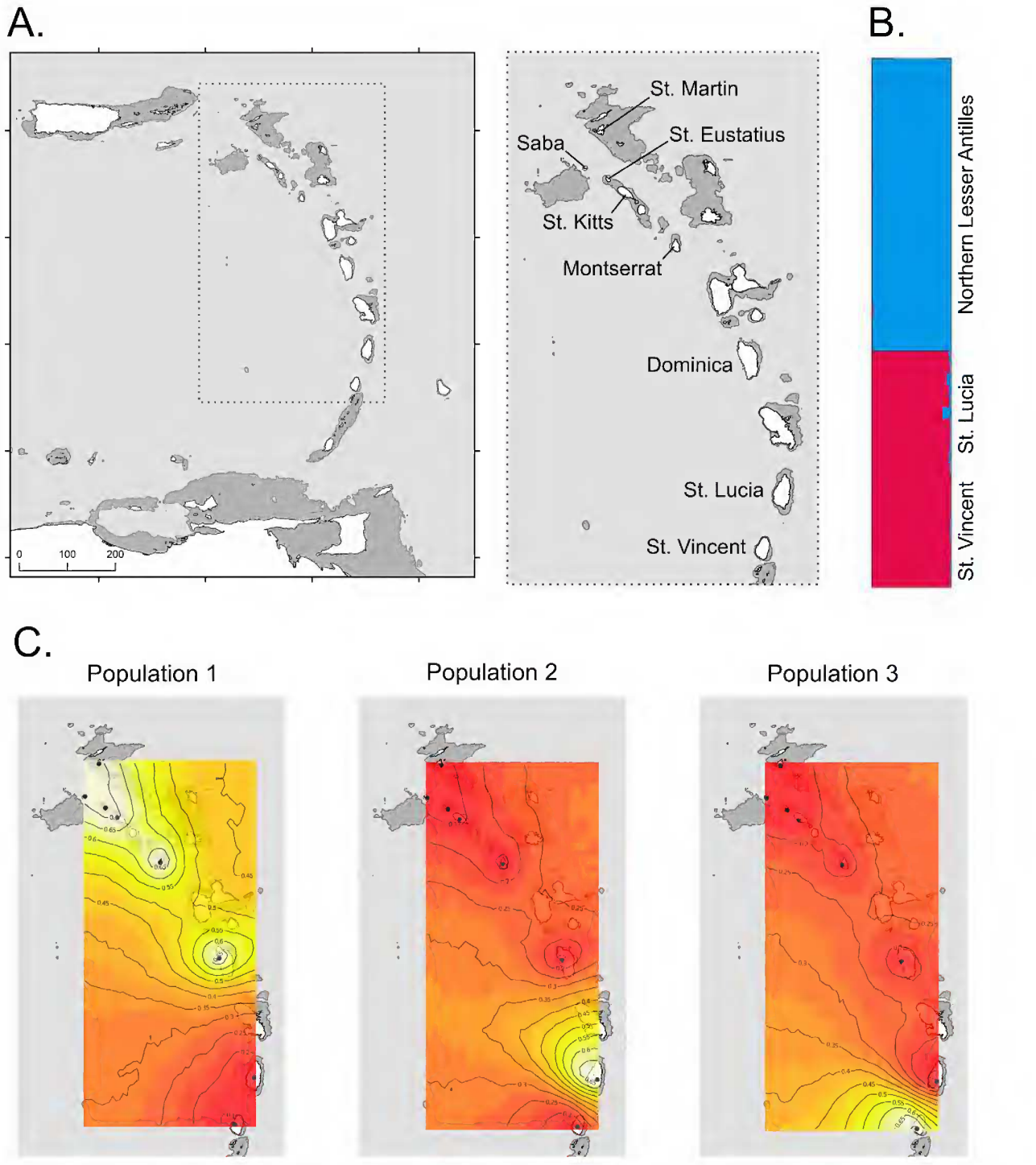

Figure 3. (A) Bathymetric map of the Lesser Antilles. Dark grey shading represents potential extent of exposed land at last glacial maximum (sea levels ~130 m below current). (B) Results of STRUCTURE analyses of AFLP data. Statistical support was recovered for two groups corresponding to the northern (blue) and southern (red) Lesser Antilles. (C) Results of GENELAND analyses of AFLP data overlaid on inset from A with black dots identifying main collecting localities. Three groups were recovered, corresponding to the northern Lesser Antilles, St. Lucia, and St. Vincent. Light colors indicate high posterior probability of group membership and dark colors indicate low posterior probability.

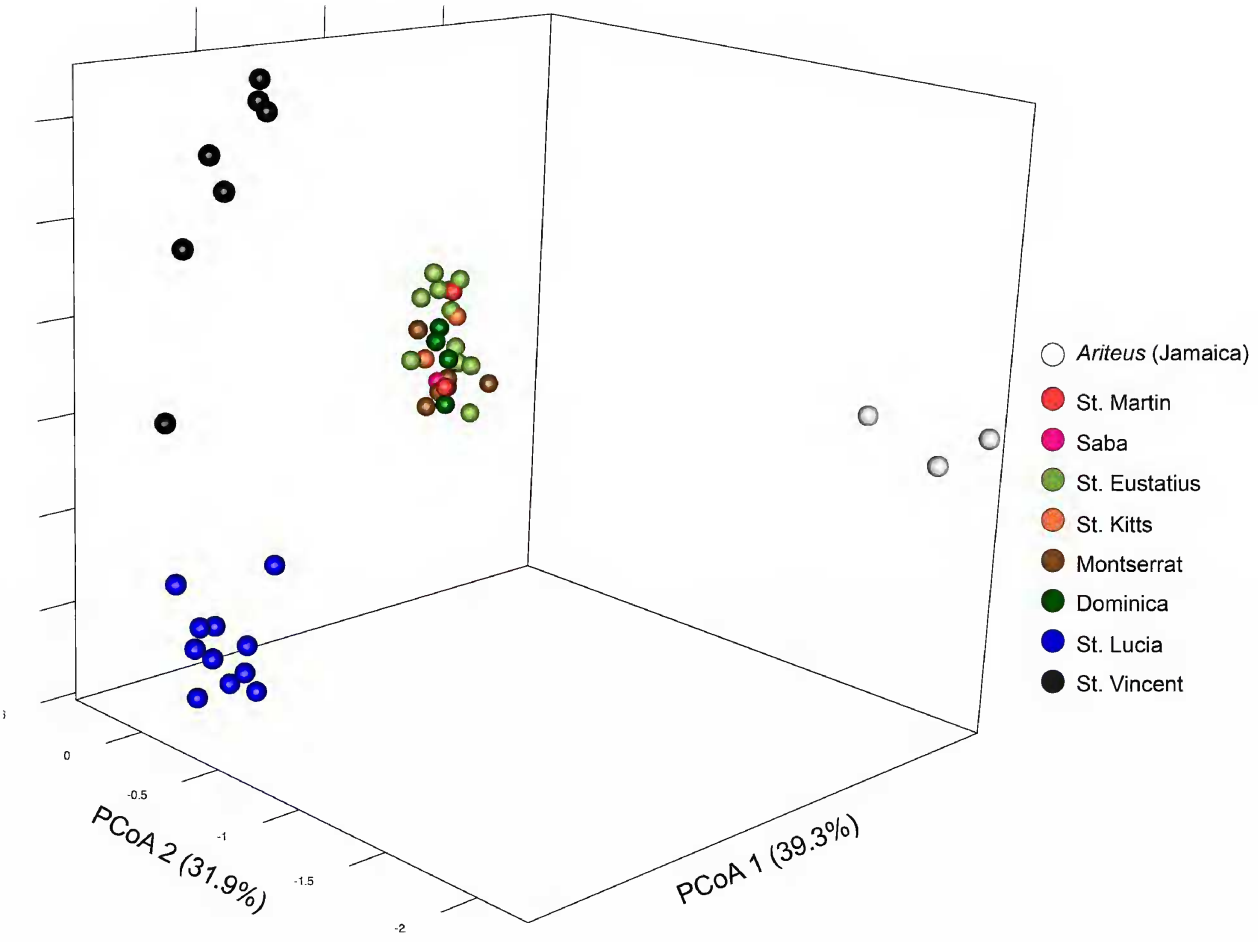

Figure 4. Principal coordinates analysis (PCoA) of 289 AFLP bands scored from *Ariteus flavescens* and *Ardops nichollsi*.

Table 2. Summary of analyses of molecular variance (AMOVA) for AFLPs in three populations of *Ardops nichollsi*. Populations defined in Figures 3C and 4. Degrees of freedom (df), sum of squares (SS), means squares (MS). Significance level ($P < 0.001$) is based on 9,999 permutations.

Source of Variation	df	SS	MS	Variation	Total variation (%)	PhiPT
Among populations	2	157.846	78.923	5.293	51%	0.512
Within populations	44	221.729	5.039	5.039	49%	

Table 3. PCA loadings along the first two principal components for *Ardops nichollsi*. If all loadings were equal, each would be (± 0.378). Only loadings higher than this value are bolded. ZB, MB, MTR, and MM had the highest component loadings on PC1, and POC was the highest on PC2 for female *Ardops nichollsi*. ZB, MTR, and MM had the highest loadings on PC1, and POC and MB were highest on PC2 for male *Ardops nichollsi*.

	Females		Males	
Character	PC 1	PC 2	PC 1	PC 2
GLS	-0.329	0.152	-0.343	0.000
CBL	-0.352	0.000	-0.345	0.170
ZB	**-0.398**	0.152	**-0.391**	0.000
POC	-0.304	**-0.932**	-0.262	**0.379**
MB	**-0.409**	0.231	-0.327	**-0.891**
MTR	**-0.434**	0.163	**-0.469**	0.151
MM	**-0.401**	0.000	**-0.464**	0.000

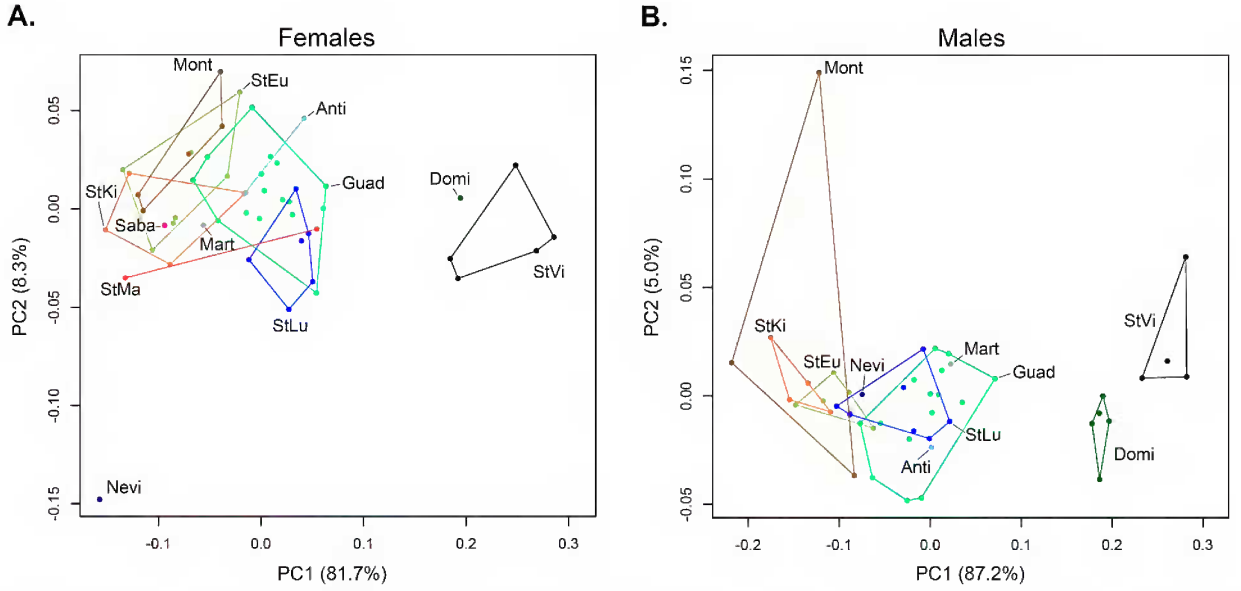

Figure 5. Principal component analysis (PCA) of seven morphological characters from (A) 51 female and (B) 47 male *Ardops nichollsi*. Polygons surround individuals from an island. The first four letters of the islands are used as labels: St. Martin (StMa), Saba (Saba), St. Eustatius (StEu), St. Kitts (StKi), Nevis (Nevi), Antigua (Anti), Montserrat (Mont), Guadeloupe (Guad), Dominica (Domi), Martinique (Mart), St. Lucia (StLu), and St. Vincent (StVi).

Table 4. Summary statistics (mean and standard deviation followed by range and sample size) of skull measurements taken from 52 female *Ardops nichollsi* from 12 Lesser Antillean islands (Appendix). Islands are in order from north to south.

Island	GLS	CBL	ZB	POC	MB	MTR	MM
St. Martin	23.58 ± 1.12	20.61 ± 1.14	15.55 ± 0.52	6.08 ± 0.35	12.56 ± 0.71	7.74 ± 0.45	10.22 ± 0.45
	22.78 – 24.37 (2)	19.80 – 21.42 (2)	15.18 – 15.92 (2)	5.83 – 6.33 (2)	12.06 – 13.06 (2)	7.42 – 8.05 (2)	9.90 – 10.54 (2)
Saba	24.14 (1)	21.19 (1)	15.98 (1)	6.10 (1)	13.03 (1)	7.75 (1)	10.42 (1)
St. Eustatius	24.11 ± 0.35	20.96 ± 0.35	15.63 ± 0.27	5.96 ± 0.19	13.03 ± 0.12	7.89 ± 0.17	10.29 ± 0.29
	23.62 – 24.59 (7)	20.28 – 21.29 (7)	15.14 – 15.90 (7)	5.61 – 6.20 (7)	12.78 – 13.15 (7)	7.72 – 8.26 (7)	9.92 – 10.70 (7)
St. Kitts	24.26 ± 0.29	21.10 ± 0.46	15.63 ± 0.25	6.07 ± 0.16	12.96 ± 0.29	7.98 ± 0.38	10.50 ± 0.35
	24.00 – 24.60 (4)	20.70 – 21.50 (4)	15.31 – 15.90 (4)	5.87 – 6.20 (4)	12.54 – 13.20 (4)	7.60 – 8.30 (4)	10.00 – 10.80 (4)
Nevis	24.30 (1)	21.40(1)	15.80 (1)	7.10 (1)	12.90 (1)	8.00 (1)	10.60 (1)
Antigua	23.38 ± 0.01	20.34 ± 0.15	15.34 ± 0.15	5.72 ± 0.21	12.47 ± 0.02	7.63 ± 0.28	9.90 ± 0.18
	23.37 – 23.39 (2)	20.23 – 20.44 (2)	15.23 – 15.44 (2)	5.57 – 5.87 (2)	12.45 – 12.48 (2)	7.43 – 7.82 (2)	9.77 – 10.03 (2)
Montserrat	24.09 ± 0.31	21.04 ± 0.24	15.76 ± 0.29	5.90 ± 0.23	12.95 ± 0.31	7.89 ± 0.15	10.25 ± 0.16
	23.60 – 24.58 (6)	20.70 – 21.34 (6)	15.40 – 16.14 (6)	5.56 – 6.10 (6)	12.60 – 13.40 (6)	7.74 – 8.10 (5)	10.02 – 10.50 (6)
Guadeloupe	23.41 ± 0.50	20.33 ± 0.40	15.33 ± 0.28	5.81 ± 0.11	12.42 ± 0.34	7.52 ± 0.15	10.14 ± 0.18
	22.80 – 24.80 (16)	19.80 – 21.50 (16)	14.88 – 15.70 (16)	5.60 – 6.00 (16)	11.90 – 13.20 (16)	7.20 – 7.80 (16)	9.75 – 10.30 (16)
Dominica	21.92 (1)	18.72 (1)	14.33 (1)	5.50 (1)	11.40 (1)	7.01 (1)	9.43 (1)
Martinique	23.50 (1)	20.70 (1)	15.90 (1)	6.00 (1)	12.60 (1)	7.70 (1)	10.50 (1)
St. Lucia	23.20 ± 0.27	20.30 ± 0.17	14.88 ± 0.22	5.91 ± 0.15	11.98 ± 0.52	7.52 ± 0.12	10.16 ± 0.17
	22.96 – 23.73 (6)	20.01 – 20.54 (6)	14.66 – 15.31 (6)	5.70 – 6.10 (6)	11.00 – 12.51 (6)	7.36 – 7.70 (6)	9.97 – 10.40 (6)
St. Vincent	21.67 ± 0.39	18.80 ± 0.28	13.74 ± 0.34	5.57 ± 0.17	11.46 ± 0.24	6.85 ± 0.13	9.02 ± 0.16
	21.02 – 22.02 (5)	18.38 – 19.04 (5)	13.35 – 14.22 (5)	5.36 – 5.75 (5)	11.26 – 11.81 (5)	6.68 – 6.98 (5)	8.86 – 9.20 (5)

Table 5. Summary statistics (mean and standard deviation followed by range and sample size) of skull measurements taken from 48 male *Ardops nichollsi* from 10 Lesser Antillean islands (Appendix). Islands are in order from north to south.

Island	GLS	CBL	ZB	POC	MB	MTR	MM
St. Eustatius	23.01 ± 0.28	20.06 ± 0.14	15.10 ± 0.23	5.90 ± 0.15	12.41 ± 0.6	7.44 ± 0.07	9.81 ± 0.22
	22.72 – 23.30 (6)	19.84 – 20.23 (6)	14.76 – 15.40 (6)	5.68 – 6.10 (6)	12.28 – 12.62 (6)	7.34 – 7.51 (6)	9.65 – 10.20 (6)
St. Kitts	23.43 ± 0.33	20.24 ± 0.27	15.42 ± 0.11	6.05 ± 0.21	12.51 ± 0.09	7.53 ± 0.13	10.01 ± 0.13
	23.00 – 23.80 (4)	19.90 – 20.55 (4)	15.30 – 15.56 (4)	5.80 – 6.30 (4)	12.40 – 12.63 (4)	7.40 – 7.70 (4)	9.90 – 10.20 (4)
Nevis	22.50 (1)	19.60 (1)	14.90 (1)	5.80 (1)	12.20 (1)	7.50 (1)	9.80 (1)
Antigua	22.62 (1)	19.32 (1)	14.42 (1)	5.78 (1)	12.28 (1)	6.93 (1)	9.30 (1)
Montserrat	23.90 ± 0.38	20.66 ± 0.44	15.64 ± 0.46	5.82 ± 0.38	11.92 ± 1.06	7.58 ± 0.14	10.06 ± 0.40
	23.58 – 24.32 (3)	20.16 – 21.01 (3)	15.12 – 16.00 (3)	5.38 – 6.07 (3)	10.70 – 12.63 (3)	7.50 – 7.75 (3)	9.60 – 10.37 (3)
Guadeloupe	22.34 ± 0.37	19.26 ± 0.27	14.76 ± 0.32	5.68 ± 0.16	12.00 ± 0.34	6.99 ± 0.14	9.59 ± 0.18
	21.33 – 22.80 (15)	18.87 – 19.80 (15)	14.21 – 15.30 (15)	5.40 – 6.00 (15)	11.48 – 12.50 (15)	6.70 – 7.20 (15)	9.30 – 10.00 (15)
Dominica	20.81 ± 0.16	17.96 ± 0.16	13.70 ± 0.28	5.44 ± 0.08	11.37 ± 0.14	6.43 ± 0.08	8.60 ± 0.11
	20.64 – 21.04 (5)	17.80 – 18.18 (5)	13.43 – 14.13 (5)	5.31 – 5.52 (5)	11.20 – 11.58 (5)	6.30 – 6.50 (5)	8.47 – 8.76 (5)
Martinique	22.40 (1)	18.90 (1)	14.60 (1)	5.50 (1)	11.50 (1)	7.00 (1)	9.60 (1)
St. Lucia	22.55 ± 0.33	19.48 ± 0.23	14.76 ± 0.25	5.80 ± 0.11	12.13 ± 0.22	7.06 ± 0.17	9.66 ± 0.32
	22.20 – 23.12 (7)	19.12 – 19.77 (7)	14.45 – 15.16 (7)	5.66 – 6.00 (7)	11.76 – 12.44 (7)	6.87 – 7.40 (7)	9.19 – 10.09 (7)
St. Vincent	20.49 ± 0.24	17.76 ± 0.19	12.99 ± 0.35	5.48 ± 0.18	10.70 ± 0.25	6.25 ± 0.07	8.32 ± 0.09
	20.27 – 20.82 (5)	17.57 – 17.93 (4)	12.61 – 13.54 (5)	5.30 – 5.77 (5)	10.34 – 10.95 (5)	6.15 – 6.34 (5)	8.21 – 8.44 (5)

TAXONOMIC REVIEW

Our evaluation of the morphologic and molecular variation in *Ardops nichollsi* leads to the recognition of an undescribed subspecies. However, we do not have complete datasets for all known populations of *Ardops nichollsi*; therefore this is not a comprehensive taxonomic review. This review will show where future data can be placed to complete this work. The last revision of bats of the genus *Ardops* was by Jones and Schwartz (1967) based on 37 specimens from seven of the Lesser Antillean islands, whereas we had 100 specimens from 12 islands available for study. Jones and Schwartz (1967) recognized a single species with five subspecies, one of which they described as new.

Family Phyllostomidae Gray, 1825
Subfamily Stenodermatinae Gervais, 1856
***Ardops nichollsi vincentensis* R. J. Larsen, Genoways, and Baker, new subspecies**

Ardops nichollsi luciae Jones and Schwartz, 1967, Proceedings of the United State National Museum, 124(3634):9–10, report of a single specimen from "St. Vincent: no specific locality."

Holotype.—Adult male, with skin, skull, and tissue samples (TK 144588). TTU 105628, from Colonarie River, 1 km S, 2.4 km W South Rivers, 248 m, Charlotte Parish, island of St. Vincent, St. Vincent and the Grenadines, Lesser Antilles; obtained by Hugh H. Genoways on 29 July 2005, original number 6407A. Deposited at the Natural Science Research Laboratory, Museum of Texas Tech University, Lubbock, Texas.

Measurements of holotype.—Total length, 65; length of hind foot, 13; length of ear, 13; weight, 13.6; length of forearm, 40.7; greatest length of skull, 20.4; condylobasal length, 17.6; zygomatic breadth, 12.8; interorbital constriction, 5.5; postorbital constriction, 5.3; mastoid breadth, 10.8; palatal length, 4.6; length of maxillary toothrow, 6.3; and breadth across upper molars, 8.2.

Distribution.—Known only from the island of St. Vincent.

Diagnosis.— Specimens from St. Vincent form a statistically supported clade based on AFLP data (Figs. 3, 4); male and female *Ardops nichollsi* from St. Vincent are the smallest-sized members of the genus in cranial measurements, approached in size only by individuals of *A. n. nichollsi* from Dominica (Fig. 5, Tables 4, 5). Males possess the southern haplotype for the very conservative ZFY intronic sequence, which is shared with *A. n. luciae* (St. Lucia) and *Ariteus flavescens* (Jamaica).

Remarks.—The newly delineated subspecies is distinguished from other populations by both molecular and morphologic characteristics. Although males from St. Vincent have the southern haplotype of the ZFY intronic sequence (Table 1), males and females have a combination of AFLPs that distinguish them at a molecular level from other subspecies of *Ardops nichollsi* (Figs. 3, 4; Table 2). Morphologically this new subspecies needs only comparison with the geographically adjacent population of *A. n. luciae* from St. Lucia. In six of the seven cranial measurements for males (except POC) and five of seven for females (except POC and MB) there is no overlap in the range of variation of these two taxa (Tables 4, 5). The principal component analyses (Fig. 5, Table 3) confirm this relationship, with *A. n. vincentensis* at the furthest right position along PC 1 indicating these bats had the smallest skulls in our study. The one taxon that approaches the position of the sample of *A. n. vincentensis* is *A. n. nichollsi* from Dominica, but their projections do not overlap and the cranial measurements confirm this relationship (Tables 4, 5). Although our study only included a single female from Dominica, the measurements for this individual are larger and outside the range of variation for three cranial measurements of females from St. Vincent (ZB, MTR, and MM; Table 4). The ranges of measurements for samples of males from the two islands do not overlap for two cranial measurements (MB and MM; Table 5) with those from Dominica being the larger. Although these two taxa are close morphologically, they differ at the molecular level and are separated geographically by two intervening islands (St. Lucia and Martinique; Fig. 1).

Jones and Schwartz (1967) had only a single male from St. Vincent available for study and it had a fragmentary skull and both forearms broken. Based on this limited material, they surmised, "bats on St.

Vincent may be smaller than those of any described race of *A. nichollsi*." Because of this limited information, however, they assigned the specimen tentatively to *A. n. luciae*. We have surveyed other Lesser Antillean islands to the east and south of St. Vincent for bats, but *Ardops* was not recovered from any of these islands, including Barbados (Genoways et al. 2011), The Grenadines (Genoways et al. 2010), and Grenada (Genoways et al. 1998). Given that bats of the genus *Ardops* appear to have a geographic distribution that follows Koopman's Line (Genoways et al. 2010), we do not expect members of the genus to be found on The Grenadines or Grenada. Genoways et al. (2011) stated: "it is our working hypothesis that the relatively young geological age of Barbados and the distance separating Barbados from neighboring islands have dually contributed to the small chiropteran fauna of Barbados." Among the species of bats "missing" from Barbados was *Ardops nichollsi*, which we do not expect will be documented from there.

Etymology.—It is our pleasure to name this unique new subspecies, *vincentensis*, in recognition of its home on the beautiful island of St. Vincent.

Specimens examined [Type Series](10).—ST. VINCENT: Charlotte Parish: Colonarie River, 1 km S, 2.4 km W South Rivers, 13°14′10.4″ N, 61°09′52.7″ W, 248 m (28 July 2005: male, TK 144593, TTU 105632; male, TK 144588, TTU 105628 [holotype]); Golden Grove, 1.5 km N, 2.7 km W Mesopotamia, 13°11′58.3″ N, 61°11′32.7″ W, 410 m (26 May 2006: male, TK 128314, TTU 105316; female, TK 128317, TTU 105319); La Soufriere Trailhead, 3.7 km W Orange Hill, 13°19′0.2″ N, 61°09′9.5″ W, 420 m (1 June 2006: female, TK 128445, TTU 105367). St. Andrew Parish: Parrot Lookout, Vermont Nature Trail, 2.3 km N, 1.75 km E Vermont, 13°13′20.2″ N, 61°12′43.4″ W, 496 m (1 August 2005: male, TK 144661, TTU 105758; female, TK 144663, TTU 105760; female, TK 144670, TTU 105767); Mt. St. Andrew, 0.35 km S, 3 km E Pembroke, 13°11′12.6″ N, 61°13′07.0″ W, 501 m (4 June 2006: male, TK 128500, TTU 105479). St. David Parish: Morgan Woods, 0.4 km N, 2.4 km E Richmond, 13°18′28.9″ N, 61°12′27.9″ W, 523 m (27 May 2006: female, TK 128334, TTU 105524). Specimens are in fluid, with skulls removed, and tissue samples (TK).

***Ardops nichollsi nichollsi* (Thomas, 1891)**

Stenoderma nichollsi Thomas, 1891, Annals and Magazine of Natural History, series, 6, 7:529.

Holotype.—Adult female in fluid with skull removed. BMNH 91.5.14.4, from an unknown locality on Dominica, Lesser Antilles; obtained by H. A. A. Nicholls.

Measurements of holotype.—Total length, 58; length of ear, 12; length of forearm, 45.7; greatest length of skull, 22.2; condylobasal length, 18.7; interorbital constriction, 6.7; postorbital constriction, 5.7; palatal length, 4.8; and length of maxillary toothrow, 7.0.

Distribution.—Known only from the island of Dominica.

Remarks.—Thomas (1891) described this new species based on a single female with a slightly damaged skull from the island of Dominica as a new species of genus *Stenoderma*, but Miller (1906) later created the new genus *Ardops* to include the Lesser Antillean taxa of this group.

The nominate subspecies is characterized by grouping with northern populations of *A. nichollsi*, in which males possess the northern haplotype of ZFY intronic sequence (Table 1), and in which AFLP analyses confirm a statistically supported group confined to the Lesser Antilles from Dominica northward (Figs. 3, 4). On the other hand, the Dominican population can be distinguished from the other northern taxon, *montserratensis*, by its overall small cranial size. In individual cranial measurements, the values for the two taxa do not overlap in five measurements for males and all seven measurements for females (Tables 4, 5). PC1 reveals this relationship with *A. n. nichollsi* to the right of the projection, and all other populations except *A. n. vincentensis* to the left of the projection (Fig. 5).

It is not possible to fully assess the relationship of *A. n. nichollsi* with *A. n. koopmani* from Martinique because of the lack of data for the latter, but comparing cranial measurements, the range of variation in males from Dominica falls below the size of the male from

Martinique for five measurements (except POC and MB), whereas the female from Dominica is smaller in all measurements than the female from Martinique (Tables 4, 5). Based on this information, *A. n. koopmani* is likely distinct, at least at the subspecific level, from *A. n. nichollsi*. The relationship of *A. n. nichollsi* to the newly described *A. n. vincentensis* is discussed in the account for the latter subspecies.

Ardops nichollsi koopmani Jones and Schwartz, 1967

Ardops nichollsi koopmani Jones and Schwartz, 1967, Proceedings of the United States National Museum, 124(3634):11.

Holotype.—Adult female in fluid with skull removed. AMNH 213951, from near Balata, Fort-de-France, Martinique, France, Lesser Antilles; obtained by Harry Beatty and Peter Martin on 18 March 1967, original no. 656.

Measurements of holotype.—Total length, 65; length of hind foot, 14; length of ear, 16; length of forearm, 50.1; greatest length of skull, 23.5; condylobasal length, 20.7; zygomatic breadth, 15.9; interorbital constriction, 7.0; postorbital constriction, 6.0; mastoid breadth, 12.6; palatal length, 5.2; length of maxillary toothrow, 7.7; and breadth across upper molars, 10.5.

Distribution.—Known only from the island of Martinique.

Remarks.—Because we lacked tissues from members of this subspecies, we were unable to perform analyses of ZFY intronic sequence or AFLPs, leaving us only limited morphologic data to assess the relationships of *A. n. koopmani*. Jones and Schwartz (1967) in their original description of *A. n. koopmani* examined four individuals and presented the cranial measurements of the same two individuals presented in our Tables 4 and 5. Jones and Schwartz (1967) stated: "*Ardops nichollsi koopmani* differs from populations of the species on adjacent islands (*A. n. nichollsi* to the north on Dominica and *A. n. luciae* to the south on St. Lucia) in being considerably larger." Other characteristics that they cited include well-developed sagittal crest, relatively narrow skull, and narrow molariform teeth. Examination of the PCA (Fig. 5) reveals that the specimens from Martinique fall with a group of bats with large-sized skulls from St. Lucia and islands from Guadeloupe northward. Compared to Dominica, the male from Martinique falls above the range of variation for five measurements and within for POC and MB (Table 5), whereas the female from Martinique is larger in all measurements (Table 4); compared to St. Lucia, the Martinique male falls within the range for four measurements and below the range for CBL, POC, and MB (Table 5), whereas the Martinique female falls within the range for only three measurements and above the range for CBL, ZB, MB, and MM (Table 4).

These results indicate that specimens of *A. n. koopmani* are larger than the individuals of *A. n. nichollsi* from Dominica, which is in agreement with the PCA (Fig. 5) and the description by Jones and Schwartz (1967). The morphologic results for *A. n. luciae* are not so clear, however, because our male specimen from Martinique falls within or below the range of values of males from St. Lucia, whereas the female from Martinique falls within or above the range of values of females from St. Lucia. These discordant results are undoubtedly, at least in part, because of having only two individuals from Martinique to analyze (Tables 4, 5). Due to the lack of molecular data and the ambiguous morphologic results, we tentatively continue to recognize this subspecies. As future data become available, the relationship between *A. n. koopmani* and other taxa will need to be further evaluated.

Ardops nichollsi luciae (Miller, 1902)

Stenoderma luciae Miller, 1902, Proceedings of the Academy of Natural Sciences of Philadelphia, 54:407.

Holotype.—Adult female in fluid with skull removed. NMNH 110921, from an unknown locality on the island of St. Lucia, Lesser Antilles; obtained by H. S. Branch on 4 February 1901.

Measurements of holotype.—Total length, 65; length of hind foot, 12.6; length of ear, 18; length of forearm, 48.0; greatest length of skull, 23.2; condylobasal length, 20.3; zygomatic breadth, 14.8; interorbital constriction, 6.6; postorbital constriction, 5.7; mastoid breadth, 12.0; palatal length, 5.5; length of maxillary toothrow, 7.5; and breadth across upper molars, 10.4.

Distribution.—Known only from the island of St. Lucia.

Remarks.—This subspecies easily is diagnosed based on males possessing the southern haplotype of the ZFY intronic sequence, which it shares with *A. n. vincentensis* and *Ariteus flavescens* (Jamaica; Table 1), in addition to the AFLP analyses where St. Lucia specimens are isolated or grouped with St. Vincent specimens (Figs. 3, 4). The large skull size of individuals from St. Lucia, easily distinguishes them from *A. n. vincentensis* on the island of St. Vincent to the south. In six measurements for males (except POC) and five for females (except POC and MB), there is no overlap in the range of measurements of these two subspecies (Tables 4, 5). As discussed above, the relationships of *A. n. koopmani* on Martinique to *A. n. luciae* are not clear at present because of the limited data available from Martinique. If these two subspecies ultimately prove to be indistinguishable, *A. n. luciae* would be the senior synonym.

***Ardops nichollsi montserratensis* (Thomas, 1894)**

Stenoderma montserratense [sic] Thomas, 1894, Proceedings of the Zoological Society of London, 1894:132–133.

Ardops annectens Miller, 1913, Proceedings of the Biological Society of Washington, 26:33.

Holotype.—Adult male in fluid with skull removed. BMNH 94.1.9.1, from an unknown locality on the island of Montserrat, Lesser Antilles; obtained by Joseph Sturge.

Measurements of holotype.—Total length, 69; length of ear, 16.5; length of forearm, 51.5; greatest length of skull, 23.8; condylobasal length, 20.8; zygomatic breadth, 15.8; interorbital constriction, 6.9; postorbital constriction, 6.0; mastoid breadth, 10.7; palatal length, 5.1; length of maxillary toothrow, 7.5; and breadth across upper molars, 10.2.

Distribution.—Known from the islands of Antigua, Guadeloupe, Marie-Galante, Montserrat, Nevis, Saba, St. Eustatius, St. Kitts, and St. Martin/St. Maarten in the Lesser Antilles.

Remarks.—This subspecies may be distinguished from others in the species complex based on males possessing the northern haplotype of the ZFY intronic sequence (shared by *Ardops* populations from Dominica northward; Table 1) and AFLP analyses indicating the northern populations were statistically significant when compared to St. Lucia and St. Vincent (Figs. 3, 4). Therefore, based on molecular data from our study, *A. n. montserratensis* and *A. n. nichollsi* cannot be distinguished; however, based on the morphologic data the two can be easily separated as shown in the PCA (Fig. 5). In individual cranial measurements the values for the two taxa do not overlap in five measurements for males and all seven measurements for females as follows (smallest *montserratensis* vs. largest *nichollsi*, males followed by females): greatest length of skull, 21.3 vs. 20.0, 22.8 vs. 21.9; condylobasal length, 18.9 vs. 18.2, 19.8 vs. 18.7; zygomatic breadth, 14.2 vs. 14.1, 14.9 vs. 14.3; postorbital breadth, only female comparisons 5.6 vs. 5.5; mastoid breadth, only female comparisons 11.9 vs. 11.4; length of maxillary toothrow, 6.7 vs. 6.5; and breadth across upper molars, 9.3 vs. 8.8, 9.8 vs. 9.4.

Although we do not have molecular data for *Ardops* from Guadeloupe, the position of the island between Dominica and the northern Lesser Antilles leads us to believe the male bats from Guadeloupe will possess the northern haplotype for the ZFY intronic sequence and males and females will have the northern Lesser Antillean AFLP pattern. The PCA results for *A. n. annectens* reveals that both males and females clustered to the left side of PC1 overlapping broadly with samples from islands to the north of Guadeloupe and from St. Lucia (Fig. 5). These samples have individuals with an overall larger skull size, whereas those samples to the right side of the projection from Dominica and St. Vincent contain the individuals with an overall smaller skull size. The samples from Guadeloupe extended further to the right of the projection than other northern samples, but the Guadeloupe samples were clearly positioned with the northern group. Examination of Tables 4 and 5 showed that mean values for the samples from Guadeloupe had, or were among those with, the lowest mean values. Individual tree bats on Guadeloupe were slightly smaller than bats from other islands in the northern Lesser Antilles, but as the PCs showed the major morphological break was between

Guadeloupe and Dominica. These insights lead us to place *A. n. annectens*, originally described by Miller (1913) from Guadeloupe, as a junior synonym of *A. n. montserratensis*, which was described 19 years earlier by Thomas (1894) based on a specimen from the island of Montserrat.

There is a population of *A. nichollsi* on the small island of Marie-Galante situated 27 km south-southeast of Guadeloupe and 30 km northeast of Dominica, which places the population geographical between *A. n. montserratensis* and *A. n. nichollsi*. We did not have specimens from Marie-Galante available for our study, but six cranial measurements (four males and two females) were recorded by McCarthy and Henderson (1992) in their initial report of *Ardops* from the island. The range of measurements for the four males and the measurements of the two females are as follows: g reatest length of skull, 21.95–22.85, 22.5, 23.3; zygomatic breadth, 14.6–15.2, 14.65, 15.5; postorbital constriction, 5.4–5.8, 5.3, 5.8; mastoid breadth, 11.7–12.1, 12.05, 12.75; length of maxillary toothrow, 7.0–7.35, 7.55, 7.55; and breadth across upper molars, 9.2–9.65, 9.95, 10.1. All of these values, except for POC, exceed the values of our sample from Dominica (Tables 4, 5) and fall within or near the range of our sample from Guadeloupe. We assign the specimens from Marie-Galante to *A. n. montserratensis*, which makes the population the southern-most for this subspecies.

DISCUSSION

Genetic variation in Ardops nichollsi.—We compared our cyt-*b* results with a previous molecular analysis of northern Lesser Antillean *A. nichollsi* (Carstens et al. 2004) who found support for genetically distinct lineages from northern Lesser Antillean islands; however, their sample consisted of only the widespread subspecies *A. n. montserratensis* from three of these islands (St. Eustatius, St. Kitts, and Nevis). With their findings, Carstens et al. (2004) suggested this northern population of *Ardops* was the result of a single founding event and the individual island populations had completed lineage sorting. With a much broader molecular sample (10 of 13 islands and four of five subspecies), we found that the islands in the north shared mitochondrial haplotypes (suggesting incomplete lineage sorting and/or maternal gene flow, Fig. 2), whereas populations from Dominica, St. Lucia, and St. Vincent did not share mitochondrial haplotypes (suggesting complete lineage sorting). Our ZFY intron sequence data from male *A. nichollsi* indicated a northern–southern island split, where *Ardops* specimens from St. Lucia and St. Vincent share ZFY intronic sequence with *Ariteus flavescens* and those from the northern islands have their own distinct sequence (Table 1). These data could be interpreted as either the ancestral sequence for the ZFY intron has been conserved within southern Lesser Antillean *Ardops*, or alternatively, it diverged but then returned to the ancestral nucleotide sequence. Finally, our nuclear AFLP data also support the observation that there is a distinct cluster in the northern Lesser Antilles as well as two separate clusters in the south, one from St. Lucia and one from St. Vincent (Figs. 3, 4). We have identified a consistent division between northern and southern lineages with our multiple molecular marker approach (paternal, maternal, and nuclear markers), thus evidence for current and potentially distinct evolutionary trajectories within *A. nichollsi* is high. We did not have genetic samples from *annectens* (Guadeloupe, Marie-Galante) or *koopmani* (Martinique), and representatives from both islands will be needed before a complete taxonomic revision of *A. nichollsi* can be made and to determine if there are other signatures of genetic isolation.

Morphological variation in Ardops nichollsi.—Earlier authors have attempted to describe the morphological variation within *Ardops*, resulting in a complex taxonomic history for *Ardops nichollsi* (Thomas 1891, 1894; Miller 1902, 1906, 1913; Allen 1942; Hall and Kelson 1959; Jones and Schwartz 1967; Jones and Genoways 1973). These studies mainly focused on the size variation within *Ardops* and the distinctiveness of males and females, with females being significantly larger (Allen 1942; Hall and Kelson 1959; Jones and Schwartz 1967). Our data confirm cranial size variation among populations of *Ardops*, as both males and females from Dominica and St. Vincent are smaller than individuals from the other islands (Fig. 5). Interestingly, specimens of *A. nichollsi luciae* from St. Lucia are more similar in morphology to specimens from

the northern Lesser Antilles. Therefore, a taxonomic assessment of the genus based strictly on morphology would result in conflicting assemblages with respect to genetic lineages and island occurrence, and indicates that the morphological variation within *Ardops* is likely plastic and related to ecological and demographic factors.

Patterns of evolution in Ardops.—When considering the historical diversity of the short-faced bats (*Stenoderma*, *Phyllops*, and *Ariteus*) in the Greater Antilles (fossil records from Koopman and Williams 1951; Koopman 1968; Steadman et al.1984; Mancina and Garcia-Rivera 2005) and the sister relationship of *Ardops* to *Ariteus* (Genoways 2001; Baker et al. 2003; Genoways et al. 2005), it is likely that the most recent common ancestor of *Ardops nichollsi* was of Greater Antillean origin. This would suggest a stepping-stone colonization pattern of the Lesser Antilles in a general north to south direction by this *Ardops* ancestor. If this hypothesis were accurate, then it would be expected that the southernmost populations (St. Lucia and St. Vincent) would be the result of a more recent colonization; however, it appears there has been sufficient time for identifiable genetic lineages to develop, corresponding geographically to St. Lucia and St. Vincent (Figs. 2–4). This pattern of isolation was not found in the northern Lesser Antilles, where those populations may be relatively older (based on a potential Greater Antillean origin). Indeed, none of the northern Antillean island populations is strictly monophyletic and the genetic data indicate these populations likely have undergone short periods of isolation followed by periods of dispersal and subsequent gene flow (Figs. 2–4). However, the congruencies in our molecular datasets provide strong evidence of a period of enhanced geographic isolation whereby gene flow between the northern and southern Lesser Antillean islands was restricted, especially between Dominica and both St. Lucia and St. Vincent. Geographic isolation of Lesser Antillean bats during the Pleistocene epoch has been hypothesized to have contributed to other recent speciation events (Larsen et al. 2010, 2011).

Given the genetic patterns observed within *Ardops*, in combination with a Pleistocene origin of the genus (Rojas et al. 2011; Baker et al. 2012), we hypothesize that Pleistocene environmental conditions likely contributed to the presence/absence of gene flow among island populations of *Ardops*. For example, at last glacial maximum, sea levels were likely ~130 meters lower (Clark and Mix 2002; Clark et al. 2009) than contemporary levels and inter-island distances would have been reduced in most cases (Fig. 3A). The northern Lesser Antillean islands sit on several large banks and ridges (Barbuda, Saba, St. Kitts, and St. Martin banks; Genoways et al. 2007a, 2007b), much of which would have been exposed during the last glacial maximum (22,000–19,000 years ago; Clark and Mix 2002; Clark et al. 2009; Fig. 3A). Gene flow in the northern Lesser Antilles would have been facilitated by the exposed land during the last glacial maximum, while water gaps in the southern chain of islands (Fig. 3A; Hill 1905; Steadman et al. 1984; Pregill et al. 1994; Morgan 2001; Genoways et al. 2010) may have played a role in the isolation of *A. nichollsi* and reduced the potential for gene flow in this part of its distribution (supported by AFLP data, Fig. 4).

It is also important to consider the unique life history traits of *Ardops* and how these could be reflected in our data. Specifically, *Ardops* is known to be an obligate tree rooster that typically travels relatively short distances when foraging (when compared to other Caribbean bat species such as *Artibeus jamaicensis* and *Brachyphylla cavernarum*; Jones and Schwartz 1967; Jones and Genoways 1973). Our research has shown that Antillean tree bats may inhabit relatively small forest patches on each island, for example, Saba is 12 square km, but *Ardops* occupies only about four square km; St. Martin is about 85 square km, *Ardops* occupies only about 1.5 square km; Antigua is about 279 square km, but only about 22 square km, or about 8% of the island, is potential habitat for *Ardops* (Genoways et al. 2007a; Lindsay et al. 2010). Thus, fluctuations in available forest habitat would impact the viability of *Ardops* populations on each respective Antillean island. The life history traits of *Ardops* may contribute to slower recovery time once a population suffers an ecological disturbance, perhaps arising from hurricane activity, droughts, and volcanic eruptions (Pedersen et al. 2010). Additionally, smaller populations of Antillean tree bats could be subject to localized extinction events, with subsequent reinvasion from adjacent islands to maintain viable populations. These events could also contribute to the morphological similarity and low level of genetic diversity observed among the northern Lesser Antillean islands, and separation from the southern

islands. Similar patterns appear in the distribution of other volant species from multiple taxonomic groups throughout the archipelago (e.g., butterflies, *Drosophila* and birds; Scott 1972; Seutin et al. 1994; Davies and Smith 1998; Hunt et al. 2001; Davies and Bermingham 2002; Wilder and Hollocher 2003).

Finally, we have not encountered *Ardops* on low-lying islands with arid-adapted vegetation, i.e., Anguilla (59 m; Genoways et al. 2007c), Barbuda (42 m; Pedersen et al. 2007), and St. Barts (281 m; Larsen et al. 2007a). We do not expect *Ardops* to be found on these three islands, as they lack appropriate habitat for Antillean tree bats. Conversely, we have captured *Ardops* on islands with elevations of at least 250 m that typically include closed canopy evergreen seasonal forests (Beard 1949).

CONCLUSIONS

It is our hypothesis that the populations of *Ardops nichollsi* evolved primarily on the islands of the Lesser Antillean Faunal Core (Genoways et al. 2001). On these islands from Guadeloupe to St. Vincent, the populations have been differentiating at both molecular and morphologic levels. This level of differentiation was not detected in populations on the islands to the north of Guadeloupe. Collectively, our morphological and molecular data have identified several interesting macroevolutionary patterns within *A. nichollsi*. In particular, our genome scan data may provide evidence for the initial stages of speciation, with the northern Lesser Antillean populations being on a separate evolutionary trajectory with respect to southern Lesser Antillean populations. Monophyly is not observed in all datasets and thus incomplete lineage sorting may account for some of the patterns observed. Similar conflicting patterns in morphological and genetic datasets have been identified in a number of bat genera, such as *Artibeus* (Marchan-Rivadeneira et al. 2012; Larsen et al. 2013), *Eumops* (McDonough et al. 2008; Baker et al. 2009), *Platyrrhinus* (Velazco and Patterson 2008), and *Myotis* (Larsen et al. 2012). Such conflict among multi-source data can be attributed to incipient speciation events (Coyne and Orr 2004) and/or incomplete lineage sorting (Funk and Omland 2003; McGuire et al. 2007). Additional research using advanced techniques such as RAD-seq and/or whole genome sequencing are required to further explore the genetics underlying phenotypic plasticity and the speciation dynamics of *Ardops*.

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APPENDIX

Specimens used in all molecular and morphological (indicated by ♀/♂) analyses. Subspecific names follow our taxonomic review. *A. f.* = *Ariteus flavescens*; *A. n.* = *Ardops nichollsi*. An asterisk (*) indicates sequences from Carstens et al. (2004).

Species	Subspecies	Island	Museum	Catalog #	Tissue #	Cyt-b GenBank	ZFY GenBank	AFLP	Sex
A. f.	*flavescens*	Jamaica	TTU	45290	27695	KJ024702	–	A	♀
A. f.	*flavescens*	Jamaica	TTU	45291	27696	KJ024703	KJ024752	A	♂
A. f.	*flavescens*	Jamaica	TTU	45293	27701	KJ024704	KJ024753	A	♂
A. n.	*montserratensis*	St. Martin	TTU	101846	129039	KJ024719	–	A	♀
A. n.	*montserratensis*	St. Martin	TTU	101868	129062	KJ024720	–	A	♀
A. n.	*montserratensis*	Saba	TTU	101954	117541	KJ024712	–	A	♀
A. n.	*montserratensis*	St. Eustatius	–	–	HapA	*AY572329	–	–	
A. n.	*montserratensis*	St. Eustatius	AMNH	3925	–	–	–	–	♂
A. n.	*montserratensis*	St. Eustatius	TTU	102002	117570	KJ024713	–	A	♀
A. n.	*montserratensis*	St. Eustatius	TTU	102003	117571	–	–	–	♂
A. n.	*montserratensis*	St. Eustatius	TTU	101978	129126	KJ024722	–	A	♀
A. n.	*montserratensis*	St. Eustatius	TTU	101979	129127	KJ024723	KJ024759	A	♂
A. n.	*montserratensis*	St. Eustatius	TTU	101980	129110	KJ024721	–	A	♀
A. n.	*montserratensis*	St. Eustatius	TTU	110925	161562	KJ024743	KJ024761	A	♂
A. n.	*montserratensis*	St. Eustatius	TTU	110926	161563	KJ024744	KJ024760	A	♂
A. n.	*montserratensis*	St. Eustatius	TTU	110927	161564	KJ024745	KJ024764	A	♂
A. n.	*montserratensis*	St. Eustatius	TTU	110928	161565	KJ024746	–	A	♀
A. n.	*montserratensis*	St. Eustatius	TTU	110929	161566	KJ024747	–	A	♀
A. n.	*montserratensis*	St. Eustatius	TTU	110930	161567	KJ024748	–	A	♀
A. n.	*montserratensis*	St. Eustatius	TTU	110931	161575	KJ024749	–	A	♀
A. n.	*montserratensis*	St. Kitts	–	–	HapB	*AY572330	–	–	–
A. n.	*montserratensis*	St. Kitts	–	–	HapC	*AY572331	–	–	–
A. n.	*montserratensis*	St. Kitts	–	–	HapD	*AY572332	–	–	–
A. n.	*montserratensis*	St. Kitts	–	–	HapE	*AY572333	–	–	–
A. n.	*montserratensis*	St. Kitts	–	–	HapF	*AY572334	–	–	–
A. n.	*montserratensis*	St. Kitts	UNSM	27576	–	–	–	–	♀
A. n.	*montserratensis*	St. Kitts	UNSM	27579	–	–	–	–	♂
A. n.	*montserratensis*	St. Kitts	UNSM	27597	–	–	–	–	♂
A. n.	*montserratensis*	St. Kitts	UNSM	27599	–	–	–	–	♀
A. n.	*montserratensis*	St. Kitts	UNSM	27600	–	–	–	–	♀
A. n.	*montserratensis*	St. Kitts	TTU	115402	165227	KJ024751	–	A	♀
A. n.	*montserratensis*	St. Kitts	TTU	115403	165231	–	KJ024754	A	♂
A. n.	*montserratensis*	St. Kitts	NMNH	543072	–	–	–	–	♂
A. n.	*montserratensis*	Nevis	–	–	HapG	*AY572335	–	–	–

Species	Subspecies	Island	Museum	Catalog #	Tissue #	Cyt-b GenBank	ZFY GenBank	AFLP	Sex
A. n.	*montserratensis*	Nevis	–	–	HapH	*AY572336	–	–	–
A. n.	*montserratensis*	Nevis	–	–	HapI	*AY572337	–	–	–
A. n.	*montserratensis*	Nevis	UNSM	27653	–	–	–	–	♂
A. n.	*montserratensis*	Nevis	UNSM	27654	–	–	–	–	♀
A. n.	*montserratensis*	Montserrat	BMNH	94.1.9.1	–	–	–	–	♂
A. n.	*montserratensis*	Montserrat	TTU	31345	15709	KJ024709	–	A	♀
A. n.	*montserratensis*	Montserrat	TTU	31353	15711	KJ024710	–	A	♀
A. n.	*montserratensis*	Montserrat	TTU	31354	15712	KJ024711	KJ024762	A	♂
A. n.	*montserratensis*	Montserrat	TTU	101874	129171	KJ024724	–	A	♀
A. n.	*montserratensis*	Montserrat	TTU	101901	129202	KJ024725	–	A	♀
A. n.	*montserratensis*	Montserrat	TTU	110924	161582	KJ024750	KJ024763	A	♂
A. n.	*montserratensis*	Montserrat	ROM	71463	–	–	–	–	♀
A. n.	*montserratensis*	Montserrat	ROM	71467	–	–	–	–	♀
A. n.	*montserratensis*	Antigua	TTU	109090	148687	KJ024731	–	–	♀
A. n.	*montserratensis*	Antigua	TTU	115400	128180	–	–	–	♂
A. n.	*montserratensis*	Antigua	TTU	115401	128181	–	–	–	♀
A. n.	*montserratensis*	Guadeloupe	TTU	20801	–	–	–	–	♀
A. n.	*montserratensis*	Guadeloupe	TTU	20802	904128	–	–	–	♀
A. n.	*montserratensis*	Guadeloupe	TTU	20805	–	–	–	–	♂
A. n.	*montserratensis*	Guadeloupe	TTU	20806	904132	–	–	–	♂
A. n.	*montserratensis*	Guadeloupe	TTU	20808	904134	–	–	–	♂
A. n.	*montserratensis*	Guadeloupe	TTU	20809	904135	–	–	–	♂
A. n.	*montserratensis*	Guadeloupe	TTU	20820	903984	–	–	–	♀
A. n.	*montserratensis*	Guadeloupe	TTU	20821	903985	–	–	–	♀
A. n.	*montserratensis*	Guadeloupe	TTU	20822	903986	–	–	–	♀
A. n.	*montserratensis*	Guadeloupe	TTU	20823	–	–	–	–	♀
A. n.	*montserratensis*	Guadeloupe	TTU	20824	8264	–	–	–	♂
A. n.	*montserratensis*	Guadeloupe	TTU	20825	–	–	–	–	♀
A. n.	*montserratensis*	Guadeloupe	TTU	20826	903996	–	–	–	♂
A. n.	*montserratensis*	Guadeloupe	TTU	20827	–	–	–	–	♂
A. n.	*montserratensis*	Guadeloupe	TTU	20828	–	–	–	–	♂
A. n.	*montserratensis*	Guadeloupe	TTU	20829	–	–	–	–	♂
A. n.	*montserratensis*	Guadeloupe	TTU	20830	–	–	–	–	♂
A. n.	*montserratensis*	Guadeloupe	TTU	20831	–	–	–	–	♀
A. n.	*montserratensis*	Guadeloupe	TTU	20832	8256	–	–	–	♀
A. n.	*montserratensis*	Guadeloupe	TTU	20833	–	–	–	–	♂
A. n.	*montserratensis*	Guadeloupe	TTU	20834	–	–	–	–	♂
A. n.	*montserratensis*	Guadeloupe	TTU	20835	–	–	–	–	♀
A. n.	*montserratensis*	Guadeloupe	TTU	20836	–	–	–	–	♂

Species	Subspecies	Island	Museum	Catalog #	Tissue #	Cyt-b GenBank	ZFY GenBank	AFLP	Sex
A. n.	*montserratensis*	Guadeloupe	TTU	20837	–	–	–	–	♀
A. n.	*montserratensis*	Guadeloupe	TTU	20838	–	–	–	–	♀
A. n.	*montserratensis*	Guadeloupe	TTU	20839	–	–	–	–	♀
A. n.	*montserratensis*	Guadeloupe	TTU	20840	–	–	–	–	♀
A. n.	*montserratensis*	Guadeloupe	TTU	20847	–	–	–	–	♂
A. n.	*montserratensis*	Guadeloupe	TTU	20848	–	–	–	–	♀
A. n.	*montserratensis*	Guadeloupe	USNM	113498	–	–	–	–	♂
A. n.	*montserratensis*	Guadeloupe	USNM	113502	–	–	–	–	♀
A. n.	*nichollsi*	Dominica	TTU	9341	902299		–	–	♂
A. n.	*nichollsi*	Dominica	TTU	31357	15602	KJ024708	KJ024755	A	♂
A. n.	*nichollsi*	Dominica	TTU	31358	15600	–	–	–	♀
A. n.	*nichollsi*	Dominica	TTU	31359	15574	KJ024705	KJ024758	A	♂
A. n.	*nichollsi*	Dominica	TTU	31360	15576	KJ024707	KJ024756	A	♂
A. n.	*nichollsi*	Dominica	TTU	31361	15575	KJ024706	KJ024757	A	♂
A. n.	*koopmani*	Martinique	AMNH	213951	–	–	–	–	♀
A. n.	*koopmani*	Martinique	AMNH	213954	–	–	–	–	♂
A. n.	*luciae*	St. Lucia	TTU	109281	151290	KJ024732	KJ024774	A	♂
A. n.	*luciae*	St. Lucia	TTU	109282	151291	KJ024733	KJ024767	A	♂
A. n.	*luciae*	St. Lucia	TTU	109285	151306	KJ024734	–	A	♀
A. n.	*luciae*	St. Lucia	TTU	109286	151307	KJ024735	–	A	♀
A. n.	*luciae*	St. Lucia	TTU	109287	151308	KJ024736	–	A	♀
A. n.	*luciae*	St. Lucia	TTU	109288	161340	KJ024738	KJ024769	A	♂
A. n.	*luciae*	St. Lucia	TTU	109291	161343	KJ024739	KJ024770	A	♂
A. n.	*luciae*	St. Lucia	TTU	109292	151358	KJ024737	KJ024768	A	♂
A. n.	*luciae*	St. Lucia	TTU	110932	161547	KJ024740	–	A	♀
A. n.	*luciae*	St. Lucia	TTU	110933	161548	KJ024741	KJ024771	A	♂
A. n.	*luciae*	St. Lucia	TTU	110934	161549	KJ024742	KJ024773	A	♂
A. n.	*luciae*	St. Lucia	USNM	110918	–	–	–	–	♀
A. n.	*luciae*	St. Lucia	USNM	110921	–	–	–	–	♀
A. n.	*vincentensis*	St. Vincent	TTU	105316	128314	KJ024714	KJ024772	A	♂
A. n.	*vincentensis*	St. Vincent	TTU	105319	128317	KJ024715	–	A	♀
A. n.	*vincentensis*	St. Vincent	TTU	105367	128445	KJ024717	–	A	♀
A. n.	*vincentensis*	St. Vincent	TTU	105479	128500	KJ024718	KJ024765	A	♂
A. n.	*vincentensis*	St. Vincent	TTU	105524	128334	KJ024716	–	A	♀
A. n.	*vincentensis*	St. Vincent	TTU	105628	144588	KJ024726	KJ024766	A	♂
A. n.	*vincentensis*	St. Vincent	TTU	105632	144593	KJ024727	–	A	♂
A. n.	*vincentensis*	St. Vincent	TTU	105758	144661	KJ024728	KJ024775	A	♂
A. n.	*vincentensis*	St. Vincent	TTU	105760	144663	KJ024729	–	A	♀
A. n.	*vincentensis*	St. Vincent	TTU	105767	144670	KJ024730	–	A	♀